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Elevated Serum ferritin and Serum Free Iron - A novel marker for pre-diabetes Type 2 in relationship with HbA1c

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Abstract

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share common phenotype of hyperglycemia. Understanding the pathogenesis and preventing long-term complications have been major goals of research in diabetes mellitus. Emerging scientific evidences has disclosed unsuspected influence between iron metabolism and type 2 Diabetes mellitus. Present study was undertaken to assess level of serum ferritin, free iron concentration, in type 2 Diabetes mellitus patients with good and poor glycaemic control and find out correlation between serum ferritin, free iron concentrations with glycaemic control. A cross sectional study consists of 450 patients out of them 150 patients having type 2 DM with good control (Group II), 150 patients with type 2 DM with poor control (Group III) and 150 normal healthy control (Group-I) were selected. Statistically significant increase in serum ferritin and free iron concentration in group III cases compare to Group I and Group II. There was a statistically significant positive correlation between serum ferritin and free iron concentration with FBS, PP2BS and Glycated Hemoglobin HbA1c. In conclusion, serum ferritin and Serum free iron concentration was higher in patients with type 2 diabetes mellitus with poor control. Also there was a positive correlation with serum free iron concentration with impaired fasting glucose levels and poor glycaemic control. These suggest important role of iron in metabolic derangement in diabetic patients and its complications.

Keywords: Type 2 Diabetes Mellitus, serum ferritin, Serum free iron, Glycaemic control.

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Introduction

Diabetes mellitus (DM) is now one of the most common non-communicable diseases globally. It is the leading cause of death in most countries. Complications from diabetes, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness are resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society. It is a chronic, incurable, costly, and increasing but largely preventable non communicable disease which is responsible for millions of deaths annually, debilitating complications and incalculable human misery. Diabetes is undoubtedly one of the most challenging health problems in 21st century [1].

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share common phenotype of hyperglycemia [2]. Hyperglycemia not only defines the disease but is the cause of its most characteristic symptoms and long-term complications [3]. Because the development of complications is linked to the accumulation of glycation adducts in tissue proteins. The core of the issue is glycaemic control. Amongst the various markers of glycaemic control, glycated hemoglobin has now been established as the most reliable [4]. Optimal

monitoring of glycaemic control involves plasma glucose measurements and measurement of hemoglobin A1c. These measurements are complementary: the patient's glucose measurements provide a picture of short-term glycaemic control, whereas the A1c reflects average glycaemic control over the previous 2 to 3months [2]. Glycated hemoglobin is formed by the glycosylation of hemoglobin. Its value represents the glycaemic status of a person over the last two to three months. HbA1c should thus be kept to less than 7% for patients in general and to less than 6% for individual patients. A1c is the primary target for glycaemic control [5].

Iron is a major component of earth's crust, but its own chemistry limit utilization and also sets basic for its toxicity. The central importance of iron in the pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical [6]. Emerging scientific evidences has disclosed unsuspected influence between iron metabolism and type 2 diabetes. The relationship is bi-directional – iron affects glucose metabolism, and glucose metabolism impinges on several iron metabolic pathways. Oxidative stress and inflammatory cytokines influences these relationships, amplifying and potentiating the initiated events. Iron induced damage might also modulate the development of chronic diabetes complications. The extent of these influences should be tested in large scale clinical trials, searching for the usefulness and cost-effectiveness of therapeutic measurements that decrease iron toxicity.

The study of individual susceptibility and of the mechanism that influences tissue iron and damage are proposed to be valuable in anticipating and treating diabetic complications [7]. There is considerable current interest in the relationship between insulin and iron pool in the body. Insulin influences the iron uptake and storage by increasing the cell surface transferrin receptors, reciprocally iron influences the insulin activity by interfering with glucose uptake and utilization. Iron causes hyperinsulinemia by decreasing the insulin uptake and metabolism by hepatocytes. Iron in its free form i.e., in non-transferrin bound form is known to induce oxidation of biomolecules through Heber-Weiss and Fenton reactions by producing harmful hydroxyl radicals [5].

Materials and Methods

Study Design and Subjects: This study was conducted at Dhiraj General Hospital and S.B.K.S Medical College and Research Centre, Vadodara, India, between January 2011 to October 2013. The study consists of 450 subjects out of them 150 patients having type 2 diabetes mellitus with good control (Group II), 150 patients with type 2 diabetes mellitus with poor control (Group III) and 150 normal healthy control (Group-I) were selected.

Inclusion Criteria:

The subjects selected for study were grouped as follows:

Group I – Control group (n=150), consists of age and sex matched healthy subjects. They were free from any ailment which could affect the parameters under study. They were not on any medication. They were taken from general population.

Group II – Diabetes Mellitus type 2 patients with good glycaemic control (n=150), with duration less than 8 years, glycated hemoglobin (HbA1C) level less than 7%. They were on life style modifications and oral hypoglycaemic drugs and free from clinical evidence of any chronic complication of diabetes mellitus

Group III – Diabetes Mellitus type 2 patients with poor glycaemic control (n=150) This group consisted of patients with type 2 Diabetes mellitus with duration more than 8 years, Glycated hemoglobin (HbA1C) level more than 7%. They were on life style modifications, oral hypoglycaemic drugs, insulin or combination of all three and associated with one or more chronic complication of diabetes mellitus for e.g. diabetic nephropathy, diabetic retinopathy, heart disease, diabetic neuropathy.

Exclusion Criteria:

The patients with type 1 diabetes mellitus, hemolytic anemia, iron deficiency anemia, hemoglobin variants, pregnancy, hepatic disease and infectious diseases like tuberculosis, sarcoidosis were excluded from this study.

The study was approved by institutional ethical committee and written informed consent was obtained from all the study subjects after explaining the objectives of the study.

Questionnaire and Data Collection: A questionnaire was specifically designed to obtain information which helps to select individuals according to the selection criteria of the study. The questions also focused on socio demographic data (age, sex) and background characteristics of diabetes (duration and type of diabetes mellitus, mode of anti-diabetic therapy, any complication). About 2 ml of venous blood was collected in fasting, post prandial (FBS & PP2BS) estimation. Serum separated within half an hour by centrifugation at 2000 rpm and stored at 2-8°C temperature till analysis was done. Blood sugar estimated by Glucose Oxidase – Peroxidase (GOD-POD) enzymatic end point method. (Kit: Manufatured by Siemens) [12]. Glycated hemoglobin (HbA1C) concentration was measured by immunoturbidimetric method (Kit: Manufactured by Agappe diagnostics Mispa-I) [13]. Serum free iron concentration was done by

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chromazurol and acetyl trimethyl ammonium bromide photometric colorimetric test. Fasting C-peptide levels by competitive enzymatic immunofluroscence method on TOSOH AIA-360 manufactured by Japan.

All biochemical investigation performed on fully automated random access analyzer Erba EM200 Transasia and Misapa I series Nephlometry principle for HbA1c and serum ferritin estimation by sandwich Immunoassay method. Hemogram and Urine examination were done in pathology laboratory. Fundoscopy and Electrocardiogram were done in respective department.

Statistical analysis: Statistical analysis was performed using SPSS 16.0. Significance in differences between mean values was assessed from independent t test. Strength of association between two variables was judged by Pearson's correlation analysis, whereas significance of dependence and predictive values analyzed by linear regression study. Chi-square test used for comparison in two groups for qualitative data. Comparison of significance of difference of average of the three study groups was done using analysis of variance (ANOVA) technique with Tukey's correction for multiple comparisons. P value <0.05 is considered to be statistically significant.

Results

Table 1: Serum free iron concentration between Group I (Healthy control) and Group II cases (Type 2 DM with good glycaemic control).

Patients	Number	Mean serum free iron µg/dl	Mean serum ferritin ng/ml	P value
Group-1	150	104.7	186.4	P<0. 3042
Group-2	150	111.36	240.3	

As shown in table 1, 2 and 3 mean serum free iron concentration in Group-I, Group II and Group III was 104.70, 111.36 and 223.46 µg/dl respectively. As shown in table 1, 2 and 3 mean serum ferritin concentration in Group-I, Group II and Group III was 186.4, 240.3 and406.5µg/dl respectively .The difference between mean serum ferritin, free iron concentration between group 1 and group 3 as well as between group 2 and group 3 is statistically highly significant (with p value <0.001). But the difference between group 1 and group 2 is not significant (p value is 0.3042).

Table 2: Serum free iron concentration between Group I (Healthy control) and Group III cases (Type 2 DM with poor glycaemic control)

Patients	Number	Mean serum free iron µg/dl	Mean serum ferritin ng/ml	P value
Group-1	150	104.7	186.4	P<0.0001
Group-3	150	223.46	406.5	P<0.0001

Table 3: Serum free iron concentration between Group II (Type 2 DM with good glycaemic control) and Group III cases (Type 2 DM with poor glycaemic control)

Patients	Number	Mean serum iron µg/dl	Mean serum ferritin ng/ml	P value
Group-2	150	111.36	240.6	P<0.0001
Group-3	150	223.46	406.5	P<0.0001

Table 4: Prevalence of increased serum free iron and serum ferritin concentration in study groups (in %)

	Group-1 (n=150)	Group-2 (n=150)	Group-3 (n=150)
Normal serum free iron concentration	97	89	14
Normal serum ferritin concentration	92	85	09
Elevated serum free iron concentration	03	11	86
Elevated serum ferritin concentration	08	15	91

Discussion

Present study was undertaken to assess level of serum free iron and serum ferritin concentration in type 2 diabetes mellitus patients with good and poor glycaemic control and find out correlation between serum free iron concentrations, serum ferritin with glycaemic control. It was carried out in Dhiraj General Hospital and S.B.K.S Medical College, Vadodara. A cross sectional study consists of 450 patients out of them 150 patients having type 2 diabetes mellitus with good control (Group II), 150 patients with type 2 diabetes mellitus with poor control (Group III) and 150 normal healthy control (Group-I) were selected.

As shown in table 4, the prevalence of increased serum ferritin concentration among Group II and Group III cases is 15% and 91% respectively.

The prevalence of increased serum free iron concentration among Group II and Group III cases is 11% and 86% respectively. The Group I patients had 03% cases with increased value of serum free iron concentration and elevated serum ferritin up to 08 % cases. Table 5 and 6 shows positive correlation between serum free iron and serum ferritin concentration with Glycated hemoglobin (GHbA1), fasting blood glucose (FBS) and postprandial blood glucose (PP2BS) and these values are statistically significant (p<0.0001)

Table 5: Correlation between serum free iron concentration (µg/dl) and GHbA1 (%), FBS (mg/dl) and PP2BS (mg/dl).

		GHbA 1c %	FBS (mg/dl)	PP2BS(mg/dl)
Serum free iron concentration µg/dl	Sample size	450	450	450
	Correlation coefficient r	0.690	0.6500	0.697
	Significance of p value	<0.0001	<0.0001	<0.0001

Table 6: Correlation between serum ferritin (ng/ml) and GHbA1 (%), FBS (mg/dl) and PP2BS (mg/dl).

		GHbA1c % (n=450)	FBS (mg/dl) (n=450)	PP2BS (mg/dl) (n=450)
Serum ferritin ng/ml.	Correlation coefficient r	0.770	0.7299	0.709
	Significance of p value	<0.0001	<0.0001	<0.0001

In our study, we found mean serum free iron concentration in Group III, Group II and Group I were 223.36µg/dl, 111.36µg/dl and 104.70µg/dl respectively. Mean serum ferritin concentration in Group III, Group II and Group I were 406.5µg/dl, 240.3µg/dl and 186.4 0 µg/dl respectively. Statistically significant increase in free iron concentration in group III cases (p<0.0001) compare to Group I and Group II. However we did not observe increase in free iron in group II cases that were in good glycaemic control. The prevalence of increased serum free iron concentration in Group III and Group II were 86% and 11% respectively whereas in Group I was only 3%, The prevalence of increased serum ferritin concentration in Group III and Group II were 91% and 15% respectively whereas in Group I was only 08%There was a significant positive correlation between free iron concentration, serum ferritin and FBS, PP2BS and Glycated Hemoglobin which measures short and long term glycaemic control in type 2 diabetes mellitus patients. The serum ferritin was significantly correlated with the fasting C-peptide in type -2 diabetics and healthy controls so serum ferritin could be marker of not only glucose hemostasis but also some components of insulin resistance syndrome in both diabetic and control subjects. These relationship is statically significant (p value<0.0001). Jeevan K Shetty et al has shown relationship between free iron and glycated hemoglobin in uncontrolled type 2 diabetes patients associated with complications [8]. S.P.Wolff studied free radicals, transitional metals and oxidative stress in the etiology of diabetes and its complications [10]. Zia A. Khan et al has studied glucose-induced regulation of novel iron transporters in vascular endothelial cell dysfunction and also shown increased iron indices and its association with the development of diabetes and its complications [11].

ManojKar and A.S.Chakraborti has studied Effect of glycosylation on iron-mediated free radical reactions of hemoglobin and demonstrated that H_2O_2 induced iron release is more from HbA1c than that from non glycosylated hemoglobin (HbA0). In the presence of H_2O_2, HbA1c degrades arachidonic acid and deoxyribose more efficiently than HbA0, which suggests that iron release is more with HbA1c compared to HbA0. Increased rate of oxidation of HbA1c in the presence of nitro blue tetrazolium is indicated by an increase in methemoglobin formation. HbA1c exhibits less peroxidase activity than HbA0. These findings on glycosylation-induced functional properties of hemoglobin suggest a mechanism of increased formation of free radicals and oxidative stress in diabetes mellitus [12]. Iron is an essential nutrient with limited bioavailability. When present in excess, iron poses a threat to cells and tissues, and therefore iron homeostasis has to be tightly controlled. Iron's toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury [9]. Iron's toxicity is largely based on Fenton and Haber–Weiss chemistry, where catalytic amounts of iron are sufficient to yield hydroxyl radicals (OH•) from

superoxide (O2• ─) and hydrogen peroxide (H2O2), collectively known as "reactive oxygen intermediates" (ROIs).

Fe^{2+} + H2O2 Fe^{3+} + OH ─ + $OH^{\cdot}$ (Fenton Reaction)

Fe^{3+} + O2 ─ Fe^{2+} + O2 Net reaction:

H2O2 + O2 ─ OH ─ + $OH^{\cdot}$ + O2 (Haber-Weiss Reaction) In this milieu, redox active iron catalyzes the generation of not only hydroxyl radicals, but also of organic reactive species, such as peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), thiyl (RS), or thiyl-peroxyl ($RSOO^{\cdot}$) radicals.

Fe^{2+} + ROOH Fe^{3+} + OH ─ + $RO^{\cdot}$
Fe^{3+} + ROOH Fe^{2+} + H^{+} + $ROO^{\cdot}$
RSH + $OH^{\cdot}$ $RS^{\cdot}$ + H2O
RSH + ROO $^{\cdot}$ $RS^{\cdot}$ + ROOH
$RS^{\cdot}$ + O2 $ROO^{\cdot}$

Free radicals are highly reactive species and maypromote oxidation of proteins, peroxidation of membrane lipids, and modification of nucleic acids. An increase in the steady state levels of reactive oxygen (and nitrogen) species beyond the antioxidant capacity of the organism, called oxidative (and nitrosative) stress, is encountered in many pathological conditions, such as chronic inflammation, ischemia–reperfusion injury, or neuro-degeneration. Excess of redox active iron aggravates oxidative (and nitrosative) stress and leads to accelerated tissue degeneration. This is evident in disorders of hereditary or secondary iron overload [7]. Interacting pathways linking glucose and iron metabolism. Insulin influences iron metabolism. Insulin is known to cause a rapid and marked stimulation of iron uptake by fat cells, redistributing transferrin receptors from an intracellular membrane compartment to the cell surface. Insulin is also responsible for the increased ferritin synthesis in cultured rat glioma cells. Importantly, transferrin receptors have been shown to co localize with insulin-responsive glucose transporters and insulin-like growth factor II receptors in the microsomal membranes of cultured adipocytes, suggesting that regulation of iron uptake by insulin occurs in parallel with its effects on glucose transport. Iron influences glucose metabolism. Reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver. Hepatic extraction and metabolism of insulin is reduced with increasing iron stores, leading to peripheral hyperinsulinemia. In fact, the initial and most common abnormality seen in iron overload conditions is liver insulin resistance. There is some evidence that iron overload also affects skeletal muscle, the main effecter of insulin action. Cytokines influence iron and glucose metabolism. Cytokines simultaneously cause an increase in transferrin receptors on the cell surface, favoring tissue deposition of iron and insulin resistance [7].

The Role of Iron in Complications of Diabetes: The importance of protein Glycation is well known in the pathogenesis of diabetic vascular complications. Transition metals also play a role in protein Glycation induced by hyperglycemia. It has been shown that glycated proteins have a substantial affinity for the transition metals, and the bound metal retains redox activity and participates in catalytic oxidation. Thus, should similar glycochelates form in vivo, reactions mediated by the chelates could be involved in the vascular complications of diabetes [6]. Role of iron in diabetic nephropathy Animal studies provide considerable evidence for the role of iron and oxidants in diabetic nephropathy. Oxidative stress from factors such as hyperglycemia, advanced glycation end products, and hyperlipidemia further contribute to the availability of intracellular iron that can generate and viciously worsen oxidative stress and renal damage. Most importantly, the pathogenic role of iron in progression is indicated by the observation that progression can be prevented either by an iron-deficient diet or chelators [6]. Role of iron in endothelial and vascular disease Pathologic mechanisms for iron in promoting vascular disease can be derived from cell culture studies, animal models, and human functional studies (vascular reactivity).

In cell culture models, the addition of NTBI to human endothelial cell cultures increases surface expression of adhesion molecules and also increases monocyte adherence to the endothelium. In human studies of end-stage renal disease patients, intravenous iron therapy has been shown to increase vascular and systemic oxidative stress, promote atherosclerosis, and increase the risk of arterial thrombosis. Further, intravenous iron has been shown to cause impaired flow-mediated dilatation in the brachial artery, a surrogate for endothelial dysfunction. Conversely, improvement in vascular reactivity after phlebotomy in patients with high-ferritin type 2 diabetes further supports these observations. Plasma NTBI measures reactive forms of iron that result in increased oxidative stress and cell injury. Alternatively, better methods of measuring excess free/catalytic iron need to be developed and validated [6]. Poor glycaemic control

causes increased Glycation of proteins, especially hemoglobin, which releases the iron in its free state. Hence increased presence of free iron in its $Fe3^{+}$ state in association with hyperglycemia might have caused decreased in the levels of protein bound thiols and increase in lipid hydro peroxides. Positive correlation between FBS and HbA1c as well as free iron and HbA1c, indicates hyperglycemia causing increased Glycation of hemoglobin and increased release of free iron from glycated proteins like hemoglobin. This makes a vicious cycle of hyperglycemia, glycation of hemoglobin and increase in levels of free iron. This increased presence of free iron pool will enhance oxidant generation leading damage to biomolecules and lead to complications. However, at present the exact nature of free iron pool in vivo is not clearly known, it needs further studies in this population with various study designs to know the catalytic action of free iron and its relation to glucose. Increase levels of serum free iron concentration and serum ferritin levels with poor glycaemic control in our study indicate important role of free iron in development diabetic complication.

Conclusions

Serum level of free iron concentration and serum ferritin was higher in patients with type 2 DM with poor glycaemic control. Also there was a positive correlation with serum free iron concentration, serum ferritin and glycaemic control. These suggest important role of iron in metabolic derangement in diabetic patients and its complications. Further a large case control studies and studies at the molecular level are required to know the role of serum free iron concentration in modifying the effect of insulin and oxidative stress in diabetes mellitus and its complication of serum free iron in patient with type 2 diabetes mellitus with poor control and positive correlation.

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References

1. A call to action on diabetes. International Diabetes federation, November 2010.
2. Kasper et al. Diabetes mellitus. In: Alvin C. Powers, editor. Harrison's Principle of Internal Medicine,16th Ed., 323; 2152-2179.
3. Thakur S, Chauhan V, Negi RC. Role of HbA1C in Diabetes Mellitus. Journal of Indian Academy of Clinical Medicine (JIACM) 2009; 10:52-54.
4. Chandalia HB, Krisnaswamy PR. Glycated Hemoglobin. Current Science 2002;83:1522-1532.
5. Nitin S. HbA1c and factors other than diabetes mellitus affecting it. Singapore Med Journal 2010; 51: 616-622.
6. Swaminathan S, Fonseca VA, Alam MG, Shah SV. The role of iron in diabetes and its complications. Diabetes Care. 2007;30(7):1926-1933.
7. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes. 2002 Aug;51(8):2348-2354.
8. Shetty JK, Prakash M, Ibrahim MS. Relationship between free iron and glycated hemoglobin in uncontrolled type 2 diabetes patients associated with complications. Indian J Clin Biochem. 2008;23(1):67-70.
9. Papanikolaou G, Pantopoulos K. Iron metabolism and toxicity. Toxicology and applied pharmacology 2005;202:199-211.
10. Wolff SP. Diabetes mellitus and free radicals. British medical bulletin 2010; 49: 642-652.
11. Zia A Khan et al. Glucose-induced regulation of novel iron transporters in vascular endothelial cell dysfunction. Free radical research 2005;39: 1203-1210.
12. Kar M and Chakraborti AS. Effect of glycosylation on iron-mediated free radical reactions of hemoglobin. Current science March 2001; 80:770-773.
13. Quantitative determination of glucose by glucose oxidase peroxidase method (GOD-POD); manufactured by Siemens pvt.ltd.
14. Quantitative determination of glycated hemoglobin (HbA1c) in human blood by latex immunoturbidimetry; Mispa-I series (Nephlometry method)
15. Quantitative determination of Serum iron bychromazurolBandcetyltrimethylammonium bromide (CTMA); Human for biochemica and diagnostic MBH

